

RemarksThe Invention:

The present invention relates to improved methods and reagents for detecting the expression of cell-surface molecules, e.g., HLA-DR and CD11b, on human peripheral blood cells. Previously, accurate measurements of cell-surface expression were hindered by the dynamic nature of expression, which resulted in measurements that changed over time in an uncontrolled and unpredictable manner. The present invention results from Applicant's unexpected discovery that the expression of cell-surface molecules can be stabilized by the addition of a lysosomotropic amine.

The present application is part of a series of applications¹ containing claims to various aspects of the invention. The parent cases, all now allowed or issued, contain claims drawn to specific embodiments of the invention relating to the stabilization of particular cell-surface molecules. Applicant has filed the present continuation application to pursue broader claims relating to the stabilization of cell-surface molecules.

Amendments to the Specification:

Applicant has amended the title to better reflect the claimed subject matter in view of the amendments to the claims. Applicant has amended the specification to include a reference to the priority application and to update the status of a parent application. The amendments do not introduce new matter.

Amendments to the Claims:

Applicant has amended the claims to focus on the generic invention described in the specification at page 32, first paragraph, as discussed above. The amendments do not introduce new matter.

¹ The present application is a continuation of U.S. Serial No. 09/406,013, filed September 24, 1999, now allowed, which is a continuation-in-part of U.S. Serial No. 09/204,860, filed December 3, 1998, which issued as U.S. Patent No. 6,200,766. U.S. Serial No. 09/645,966, filed August 24, 2000, now allowed, is continuation of U.S. Serial No. 09/204,860.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is entitled “Version With Markings To Show Changes Made.”

Conclusion

Applicant requests entry of the above amendments and remarks the record.

Respectfully submitted,

2/21/02
Date

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Version With Markings To Show Changes Made

In the Specification:

The title has been amended as follows:

[Improved] Methods and Reagents for Quantitation of [HLA-DR and CD11b]
Cell-Surface Molecule Expression on Peripheral Blood Cells

The section, Cross Reference to Related Applications, has been amended as follows:

This application is a continuation of copending U.S. Patent application Serial No. 09/406,013, filed September 24, 1999, which is a continuation-in-part of [copending] U.S. Patent application serial no. 09/204,860, filed December 3, 1998, which issued as U.S. Patent No. 6,2000,766, the disclosures of both of which [is]are incorporated herein by reference in [its]their entirety.

In the Claims:

Claims 14 - 24, 26, 29-38, 46-50, 52-53, and 55-65 have been cancelled.

The following claims have been amended as indicated:

1. (amended) A method of measuring [HLA-DR] expression of a cell-surface molecule on the surface of human blood cells, comprising:
contacting a sample containing human blood cells with a lysosomotropic amine and an antibody specific for [HLA-DR]said cell-surface molecule; and then
detecting the binding of said [anti-HLA-DR] antibody to said cells.

3. (amended) The method of [either] claim 1 [or claim 2], wherein said lysosomotropic amine is selected from the group consisting of chloroquine, hydroxychloroquine, primaquine, and methylamine.

6. (amended) The method of [either] claim 1 [or claim 2], wherein said [anti-HLA-DR] antibody is labeled with a fluorophore.

9. (amended) The method of claim 8, wherein said fluorophore is conjugated to said [anti-HLA-DR] antibody at a defined molar ratio.

11. (amended) The method of [either] claim 1 [or claim 2], wherein said antibody binding is detected flow cytometrically.

12. (amended) The method of claim 11, wherein said lysosomotropic amine is chloroquine and said [anti-HLA-DR] antibody is conjugated to PE.

13. (amended) The method of claim 12, wherein said [anti-HLA-DR] antibody is conjugated to PE at a molar ratio of 1:1.

39. (amended) A composition for flow cytometric measurement of [HLA-DR]a cell-surface molecule on human peripheral blood cells, comprising:
a fluorophore-conjugated [anti-HLA-DR] antibody specific for said cell-surface molecule, and
a lysosomotropic amine.

51. (amended) A kit for flow cytometric measurement of [HLA-DR]a cell-surface molecule on the surface of peripheral blood cells, comprising:
a composition according to claim 39, and
an erythrocyte lysing composition.